Chapter 32

MECHANISM OF NOISE-INDUCED HEARING LOSS AND OTOPROTECTIVE STRATEGIES

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Despite continuing advances in hearing conservation over the past several decades, noise-induced hearing loss (NIHL) remains a major cause of deafness in industrialized nations. Significant reasons for the persistence of this problem include steadily increasing noise levels and inherent limitations to environmental engineering solutions and personal hearing protection devices (HPDs). Recently, insights into the molecular mechanisms of noise-induced cochlear injury have led to new treatment strategies that render the cochlea more resistant to noise as well as enhance the recovery of noise-injured cochleae. Combining this therapeutic approach with current hearing conservation practices will improve treatment of and protection from acute NIHL. Future work may focus on cochlear sensory cell regeneration to treat and restore hearing even after chronic hearing losses.

The U.S. military and the National Institutes of Health (NIH) have done much to promote and standardize hearing conservation practices over the last several decades. Despite advances, NIHL is still one of the most common military disabilities, affecting an estimated 10 to 15% of the armed forces. Significant hearing threshold shift (STS) rates vary from 29% to over 70% in some at-sea Navy job specialties. STS rates tend to be even higher for aircrew, whether land or sea based. In several studies, STS rates were reported to be as high as 11% after only short periods of periodic weapons-generated noise exposure.

Besides the military, many other vocations are considered to be noise hazardous. The National Institute of Deafness and Other Communication Disorders (NIDCD) estimates that some 10 million persons in the United States have some degree of NIHL. The railroad, plumbing, carpentry, and coal-mining industries are but a few of the noise-hazardous occupations. Recreational firearm use is popular in the United States and contributes significantly to the overall problem, and NIHL contributes significantly to the 30 million individuals in the United States with hearing impairment and to the associated $56 billion annual cost. In addition, NIHL is considered to be an international problem, with some 600 million individuals worldwide estimated to be at risk primarily through occupational exposure.

Environmental engineering solutions to abate noise are a critical part of prevention and are often effective; however, they generally are impractical, economically unfeasible, or ineffective due to physical and acoustic parameters. Another important line of defense is the use of HPDs during periods of sound exposure. Lack of compliance obviously negates their effectiveness for the protection of hearing; moreover, HPDs suffer from several inherent limitations. Often, the level of noise exceeds the protective capability of the HPD, or damaging sound energy is transmitted directly through the skull, bypassing the protective device. Maintaining a comfortable and reliable acoustic fit of an HPD can be problematic. The element of surprise coupled with the need to periodically remove the device to hear also can lead to acoustic injury. NIHL can occur following even a short exposure to intense sound. Furthermore, evidence suggests that inhaled toxicants commonly found in noisy occupational environments, such as carbon monoxide, ethyl benzene, toluene, and styrene, can act additively to injure the noise-exposed inner ear. Many of the environmental- and HPD-limiting factors are difficult to overcome; hence, pharmacological-based therapies to render the cochlea more resistant to noise damage, as well as reverse or treat acute noise-induced hearing loss, may prove useful.

HOW ACOUSTIC OVEREXPOSURE DAMAGES THE COCHLEA

To develop a rational approach to pharmacological therapies for the prevention or treatment of acoustic overexposure, an understanding of the mechanisms by which noise injures the cochlea must be understood. Over the past decades, significant progress has been made in understanding the cellular and molecular mechanisms of noise injury to the cochlea. This, in turn, has led to exciting experimental data, which appear promising for the development in the near future of both protective and therapeutic agents to ameliorate the effects on sound trauma.

Cochlear injury due to excessive noise can be divided broadly into mechanical and metabolic mechanisms. With exposures of ~115 to 125 dB sound pressure level (SPL) at the ear, mechanical damage tends to predominate. This damage may include the disruption of such structures as Reissner's membrane, basilar membrane–cell junctions, damage to or loss of stereocilia bundles, and even disruption of subcellular organelles, such as the endoplasmic reticulum. Damage to hair cell–Deiters' cell junctions at the level of the reticular lamina can lead to an admixture of potassium-rich endolymph, with the perilymph surrounding the cell bodies of the outer hair cells, leading to their destruction. However, in most clinically relevant scenarios, the level of noise exposure to the ear is less than 115 dB, and the damage tends to be metabolically driven. (The remainder of this chapter will focus on metabolic causes of noise-induced injury of auditory sensory cells and how they may be ameliorated.)

The cochlea is a highly metabolically active sensory organ, which receives 0.5 mL per minute of blood flow under normal conditions, a relatively high amount of flow compared with other organs of similar size. One theory of noise-induced cochlear injury is that a consequence of acoustic overexposure is a temporary reduction in blood flow leading to a cochlear ischemia-reperfusion injury similar to that which occurs during a stroke in the brain.

Although several earlier studies have suggested that cochlear blood flow (CoBF) may increase with some excessive noise exposures, the evidence from many recent studies is that CoBF actually decreases to the cochlea during loud sound exposure. Investigations utilizing intravital microscopy, which allows for real-time, continuous and quantifiable observation of the vessels of the lateral wall of the cochlea, have demonstrated localized ischemia in response to prolonged exposure to a loud sound. In addition, researchers using laser Doppler flow meters have made measurements of blood flow in the cochlea during loud sound exposure that are consistent with the findings of the other reports, that CoBF decreases during exposure to a traumatic level of noise.

The current development of this theory is that noise provokes hypoperfusion and ischemia in the microcirculation of the cochlea, followed by reperfusion of the cochlea, and this generates reactive oxygen species (ROS) and other free radicals, as has been demonstrated,

to occur in the brain in response to a stroke. ROS have been shown to form in the cochlea after either loud sound exposure or blast trauma. It is believed that these ROS can assault the sensory cells of the cochlea, which can lead to cell death, and thus the permanent threshold shifts associated with NIHL.

Free radical molecules are characterized by the presence of unpaired electrons, making them chemically unstable and highly reactive with cellular proteins, membrane lipids, deoxyribonucleic acid (DNA), and cellular organelles. ROS are further characterized in that they contain at least one molecule of oxygen. Evidence for the production of superoxide and hydroxyl radical anions in the cochlea in the face of noise-induced ischemia-reperfusion has been published.

OXIDATIVE STRESS HYPOTHESIS FOR NOISE-INDUCED COCHLEAR INJURY

Evidence has subsequently accumulated that indicates that ROS play a major role in certain acoustic overexposure conditions. The evidence for the oxidative stress hypothesis is strongly supported by published work from several different research centers. **Fig. 32–1** depicts a summary of oxidative stress in the cochlea, depicting free radicals that may be generated (**Fig. 32–1A**), intrinsic cochlear antioxidant defenses (**Fig. 32–1B**), and the consequences of oxidative damage to hair cells when the antioxidant defenses of the auditory sensory cells are overwhelmed (**Fig. 32–1C**). Some of the intrinsic cochlear antioxidant defenses include antioxidant enzymes, heat shock proteins, trophic factors, small molecules such as vitamins C and E, and the crucial antioxidant molecule, reduced glutathione (GSH), as shown in **Fig. 32–1B**.

Acoustic overexposure leads to a modulation of antioxidant enzymes and a reduction in GSH levels within the cochlea. The generation of ROS is correlated with acoustic overstimulation in the cochlea, even after the cessation of the noise exposure. If these ROS are infused without any noise exposure into the cochlea, they produce a characteristic pattern of damage and hair cell loss just as if the cochlea has been overstimulated by a damaging level of sound. Lipid peroxidation, one of the hallmarks of oxidative stress injury within a cell, has been observed in the spiral ganglion, organ of Corti, and stria vascularis, after acoustic overstimulation. A reduction of GSH availability to the inner ear intensifies noise-induced cochlear injury. Conversely, augmenting inner ear antioxidant defenses by increasing antioxidant enzyme activity, increasing the level of available inner ear GSH, or adding exogenous antioxidant compounds can reduce

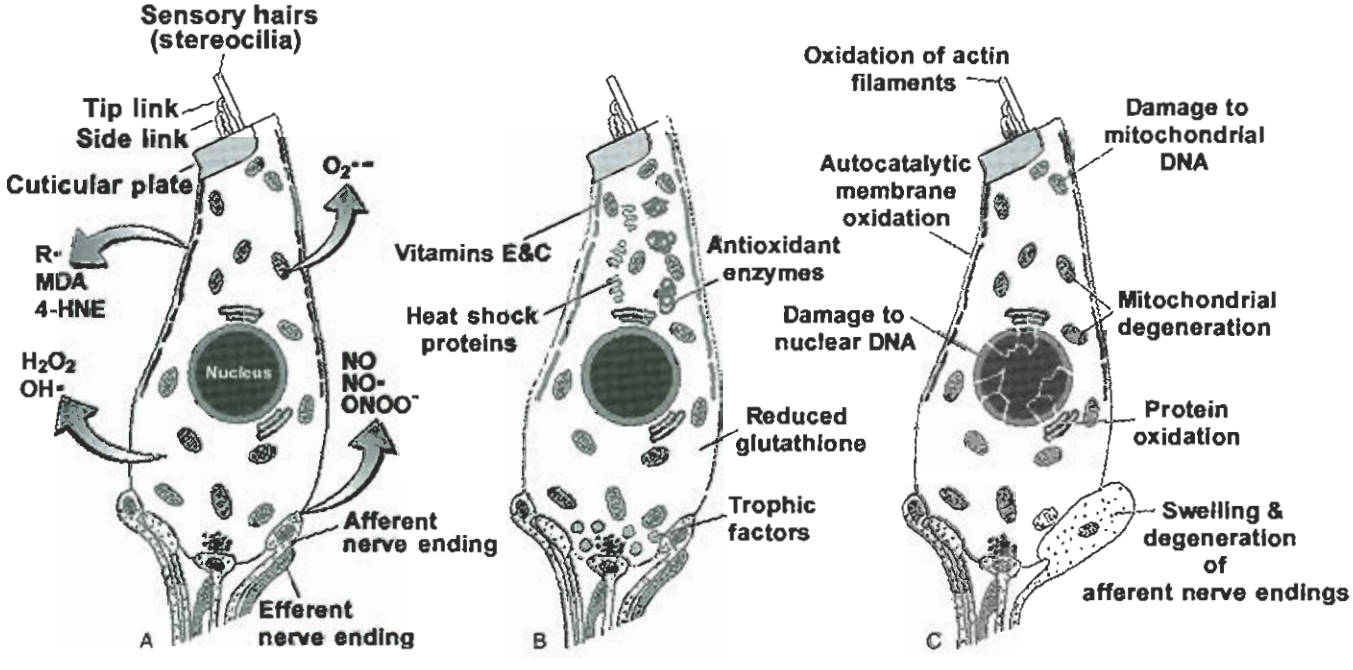


Figure 32–1 *Noise exposure causes oxidative stress injury to cochlear sensory cells. (A) The main types of free radicals and reactive oxygen species (ROS) demonstrated or postulated to be formed in the cochlea during acoustic overexposure. (B) The hair cell's defenses against oxidative stress. (C) When free radicals and ROS overwhelm the oxidative defenses of a cell, damage occurs to numerous cellular components, leading to cell injury and/or cell death. 4-HNE, 4-hydroxy-2, 3-nonenal; MDA, malondialdehyde.*

the amount of permanent hearing loss caused by noise. It has been reported that the well-known increased sensitivity to noise and other toxins of the basal region of the cochlea may in part be due to a relative weakness of antioxidant defenses [i.e., GSH in outer hair cells (OHCs) from basal regions as compared with the apical region] in this segment of the cochlear duct.

When ROS or free radicals are generated in excessive amounts, as may occur with acoustic overexposure, they overwhelm cochlear antioxidant defenses. These highly reactive compounds oxidize cell membrane lipids, intracellular proteins, and DNA, leading to injury and/or cell death, as shown in **Fig. 32–1C**. During acoustic overstimulation, ROS may be generated from a variety of sources, including the mitochondria or secondary to ischemia-reperfusion, toxic effects of release of excessive levels of glutamate, large increases in intracellular calcium, or microlesioning of the cell membrane. Excessive generation of ROS by the cell's mitochondria injures those mitochondria, which leads to further generation of ROS.

Molecules of ROS are unstable, and they interact with cell membranes, disrupting their integrity. Peroxidation of cell membranes initiates an autocatalytic chain reaction of cell membrane oxidation with the production and release of toxins such as 4-hydroxy 2,3-nonenal (HNE), an aldehyde adduct of membrane lipid peroxidation. HNE is itself a toxic molecule, and when applied to auditory sensory cells, it causes their death via apoptosis (programmed cell death). Protein oxidation may result in the loss of structurally important actin filaments, leading to the loss of functional hair bundles and the disruption of other important regulatory proteins that control maintenance of cell stability for critical ions (e.g., calcium). This can lead to further cell injury and culminate in the initiation of cell suicide; that is, apoptosis.

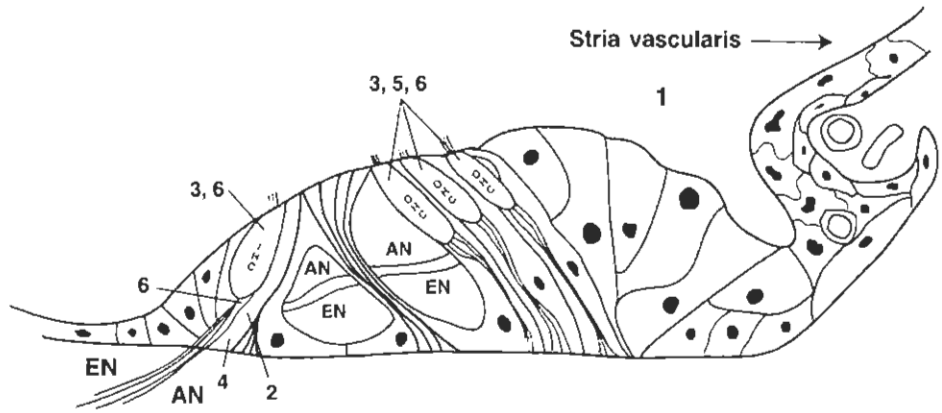
Lost hair cells are replaced by the expansion of the luminal surface area of neighboring supporting cells, leaving a scar as part of a healing process. However, these supporting cell scars that replace the auditory hair cells cannot transduce sound. It may take several days or even weeks after noise exposure until the actual loss of hair cells occurs. It is becoming apparent with narrowband noise exposures that a narrow segment of the organ of Corti correlated with the frequency of the injurious sound loses OHCs within a day or two. Then, depending on the intensity of the acoustic overexposure, waves of OHC losses occur in both basal and apical directions from the site of the initial injury over a period of days to weeks, much like the cell death that occurs in the areas of the brain (penumbra) that surround the initial site of a stroke lesion. The primary mode of OHC death in the apical- and basal-directed delayed losses appears to be apoptosis. This critical time interval can allow for a potential therapeutic window where rescue and repair could be initiated after the noise injury, but prior to the initiation of programmed cell death, thereby reducing the amount of permanent hearing loss.

ADDITIONAL GENERATORS OF OXIDATIVE STRESS DURING ACOUSTIC OVEREXPOSURE

If oxidative stress plays a major role in noise-induced cochlear injury, as current evidence suggests, then it is important to understand the potential generators of oxidative stress in more detail to formulate a rational approach to the development of effective pharmacological protection and treatment strategies. Although ischemia-reperfusion undoubtedly plays a role, evidence is accumulating that there may be other important sources of ROS and other free radicals in the noise-challenged cochlea. Postulated oxidative stress generators include mitochondrial generation of excessive ROS in cochlear cells, glutamate excitotoxicity secondary to excessive release of this neurotransmitter from afferent hair cell synapses during acoustic overexposure, GSH depletion in cochlear cells, and harmful fluxes in calcium ions within cochlear cells. It is likely that the resultant damage caused by cochlear oxidative stress is the result of multifactorial mechanisms acting in concert. This concept is summarized in **Fig. 32–2**.

Mitochondria

A major source of free radicals generated in oxidative-stressed cells is the mitochondria. Normally, 1% of the reactions of the electron transport chain yield free radicals. Under stress conditions, this percentage is increased, and when mitochondria are damaged, oxidative phosphorylation is inefficient, resulting in the production of even higher levels of ROS. One of the early subcellular changes noted in noise-stressed cochlear hair cells is mitochondrial swelling, a hallmark of mitochondrial oxidative injury. It can be seen as early as 2 to 4 hours after the onset of acute excessive noise exposure; hence there is evidence that (1) mitochondrial injury and (2) consequent enhanced levels of oxidative stress due to an uncoupling of oxidative phosphorylation are early events in the pathogenesis of acoustic trauma. In further proof, inhibition of mitochondrial self-repair intensifies



1. Ischemia reperfusion
2. Excessive glutamate release
3. Hyperproduction of ROS by mitrochondria
4. Enhanced nitric oxide synthase activity
5. GSH depletion (inhibition of cystine-glutamate antiporter)
6. Increases in intracellular calcium

Figure 32–2 Postulated generators of noise-induced oxidative stress within the cochlea. Diagram of the organ of Corti and stria vascularis depicting possible generators of oxidative stress occurring in response to acoustic overexposure. Possible sites of action: (1) ischemia-reperfusion injury may be generated in the stria vascularis by noise-induced vasoconstriction; (2) excessive glutamate release may occur at the inner hair cell afferent synapse and possibly at the small number of afferent synapses at the bases of outer hair cells (OHCs); (3) mitochondria production may lead to an abnormally high amount of reactive oxygen species (ROS) in hair cells; (4) increased nitric oxide synthase activity may lead to the production of high levels of nitric oxide and related free radicals; (5) depletion of glutathione (GSH) may occur predominantly within OHCs if the cystine-glutamate antiporter is inhibited; and (6) increases in intracellular calcium may occur in hair cells through a variety of mechanisms. AN, afferent nerve; EN, efferent nerve; IHC, inner hair cell.

noise-induced inner ear injury. In addition to being damaged by the very free radicals that the injured mitochondria overproduce, other molecular events related to intense noise may lead to mitochondrial injury.

Glutamate excitotoxicity, GSH depletion, excessive increases in intracellular calcium, and ischemia-reperfusion can all lead to mitochondrial injury, and all of these factors have been implicated in noise injury to the cochlea. The consequences of mitochondrial injury include loss of key mitochondrial molecules, such as carnitine and cardiolipin, reduced activity of cytochrome c and other important enzymes, mitochondrial "electron leak" from the electron transport chain, loss of mitochondrial membrane integrity, and eventual onset of mitochondrial-induced cell death. Mitochondrial membrane permeability (MMP) is central in the cell death process. With a sufficient degree of mitochondrial injury, the mitochondrial membranes become permeable to molecules and release respiratory enzyme molecules (e.g., cytochrome c) that activate cell death effector proteins (e.g., caspases), which is one pathway for activation of apoptosis. Programmed cell death pathways involving calpain, caspases, and JNK/c-Jun molecules also have been noted to be activated in the cochlea in response to a damaging level of noise exposure.

ROS damage can depolarize mitochondria and cause an increase in the permeability of mitochondrial membranes that leads to pore formation and release of cytochrome c from the inner mitochondrial membrane. Cytochrome c, once released from its membrane-anchored location, can then pass through the pores formed in the outer mitochondrial membrane into the cytoplasm of the ROS-damaged cell. Once cytochrome c enters the cytoplasm, it enters into the apoptosome complex, where it combines with APAF-1 (the human homolog of the *c. elegans* proapoptosis molecule CED-4) and can now activate procaspase-9 into its active form, caspase-9 (**Fig. 32–3**). Activated caspase-9 can now act on downstream procaspase molecules, such as procaspase-3, to convert them to activated effector caspases (i.e., caspase-3). It is these activated effector caspase molecules that act on apoptotic substrates within the ROS-damaged cell that cause the degradation of membrane lipids, cellular proteins, and the typical pattern of DNA degradation that forms a characteristic pattern of DNA laddering that can be seen on gels and is considered to be a sure sign of a cell's irreversible

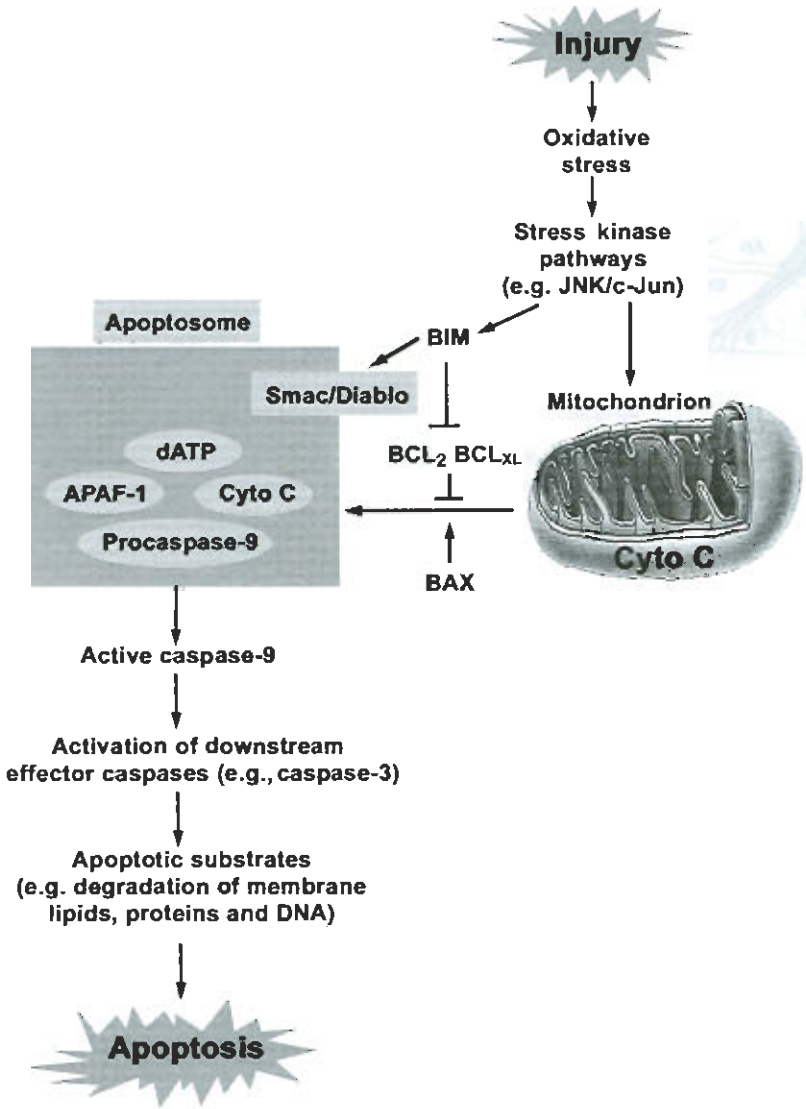


Figure 32–3 Flow diagram of a cell-death pathway showing the cascade of events from initiating injury to apoptosis of damaged inner ear sensory cells. An injury generates oxidative stress within a sensory cell, causing the generation of ROS and free radical damage that activates a stress kinase cell-death pathway (e.g., mitogen activated protein kinase) [MAPK], that involves c-Jun-N-terminal kinase (JNK) and c-Jun. JNK/c-Jun can then cause both the activation of a proapotic BH-3, only member of the Bcl-2 family (BIM) and damage to both the inner and outer mitochondrial membranes that results in the release of cytochrome c into the affected cells cytoplasm. Once in the cytoplasm, cytochrome c enters the apoptosome, where it interacts with APAF-1 and acts upon the pro- (inactive) form of an initiator (i.e., caspase-9), resulting in activated caspase-9, which then activates the inactive forms of downstream effector caspase molecules (e.g., caspase-3). Once activated, the effector caspases degrade cellular membrane lipids, proteins, and nuclear DNA, which results in the apoptotic cell death of the affected sensory cell. The Bcl-2 family of pro- and anti-apoptotic molecules all act to either inhibit (anti-) or initiate (pro-) pore formation in the mitochondrial membranes. It is the formation of pores in the mitochondrial membrane that releases the cytochrome c from the mitochrondrion into the cytoplasm. Bcl-2 and Bcl_{xl} act to inhibit pore formation (anti-apoptotic), and BIM (pro-apoptotic) acts to inhibit the mitochondrial membrane stabilizing action of Bcl-2 and Bcl_{xl}. BAX acts directly (pro-apoptotic) to form pores in the mitochondrial membranes.

commitment to apoptosis. This degradation of nuclear DNA by the caspases and other degradation enzymes (e.g., poly-ARP-ribose-polymerase [PARP] activated by caspase also cause free ends of the degraded DNA to label with the terminal uridine nick translation end-labeling (TUNEL) technique used to identify cells that are in the process of apoptosis. It is this damage to cellular proteins, lipid membranes, and nuclear DNA that is downstream of the release of cytochrome c from the ROS-damaged mitochondria that causes an irreversible commitment of oxidative stress–damaged sensory cells to elimination via apoptosis. Because of all of the cell death events that are activated by cytoplasm localized cytochrome c, the release of this respiratory chain enzyme from the ROS-damaged mitochondria into the cytoplasm is considered to be a significant step in a damaged cell's progress toward a commitment to a cell death (apoptosis) program. Members of the B cell lymphoma molecule two (Bcl-2) family of anti- and pro-apoptotic molecules play important roles in the mitochondrial release of cytochrome c into the cytoplasm of a ROS-damaged cell within the inner ear. It is the balance between the pro- and anti-apoptotic Bcl-2 molecules that either protects against or initiates the depolarization of mitochondrial membranes, formation of membrane pores, and release of cytochrome-c from the damaged mitochondria. BAX and BIM are pro-apoptotic members of the Bcl-2 family that act to enhance pore formation and to inhibit the anti-apoptotic protective effects of Bcl-2 and Bcl_{xl}, members of the Bcl-2 family. BAX directly forms pores in the mitochondrial membranes, and BIM inhibits the anti-pore forming protection of Bcl_2 and Bcl_{xl}. Bcl_2 and Bcl_{xl}, anti-apoptotic factors, act to stabilize mitochondrial membranes and prevent pore

formation, preventing the release of cytochrome c from ROS-damaged mitochondria and the downstream effects of procaspase activation to its active form as a caspase. Thus, mitochondria may act as both a source of ROS and also as the cell executioner in the face of excessive oxidative stress.

Glutamate Excitotoxicity

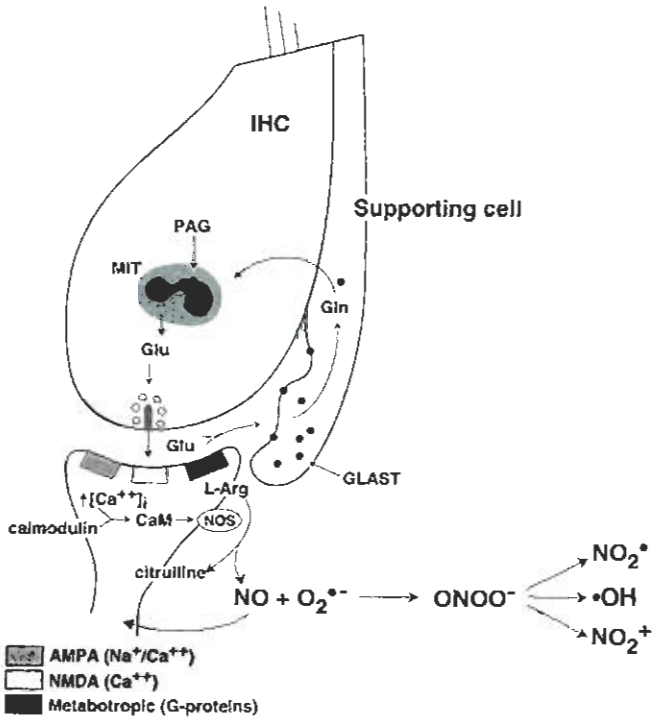
Glutamate is a major neurotransmitter between inner hair cells and afferent cochlear nerve endings of the type I spiral ganglion neurons. It has been postulated that exposure to excessive sound stimulation causes excessive synaptic glutamate concentrations to develop, which lead to overstimulation of the glutaminergic receptors, invoking metabolic cascades, which, in turn, lead to cell injury and death. Some of the potentially harmful cascades set in action by glutamate excitotoxicity may include increases in intracellular calcium with the activation of calcium-dependent calmodulin, the subsequent activation of nitric oxide synthase (NOS), resulting in excessive production of nitric oxide (NO), and the generation of related free radicals such as peroxynitrite. Other glutamate-induced processes may include activation of protein kinases; phospholipase A_2; proteases, such as calpain; and xanthine oxidase, with the subsequent generation of superoxide anions, in addition to mitochondrial injury. Looking to central nervous system glutamate excitotoxicity as an example, glutamate excitotoxicity can be divided into an early phase (up to 30 minutes) and a late phase (3–24 hours) of ROS production consequent to excessive glutamate production. The latter phase of glutamate-induced ROS production occurs as a self-propagating process in which damaged mitochondria become the source of both additional ROS production and further damage to the affected cell.

Evidence for the involvement of glutaminergic synapses in the pathogenesis of NIHL includes the fact that glutamate agonists infused into the inner ear can create an injury to the organ of Corti that is similar in histology to a lesion caused by acoustic overexposure. Also, a variety of glutamate antagonists can partially prevent hearing loss in experiments with animal models of NIHL. Organ of Corti damage produced through noise-induced release of excessive glutamate is thought to occur through a variety of different mechanisms.

At the level of the inner hair cell dendrites, excessive glutamate release is associated with a massive influx of ions and water into these dendritic terminals, leading to loss of the dendritic process. Pujol and colleagues have shown that this glutamate-initiated dendritic damage is at least partially reversible, and that the dendritic processes can be regenerated and form new synapses with the hair cells. Excessive glutamate release is also thought to cause an increase in the activity of NOS. The resulting overproduction of NO, in turn, can lead to the production of several free radicals, such as peroxynitrite, that can directly damage cellular structures. At the level of the OHC, chronic excessive glutamate exposure can lead to intracellular GSH depletion, excessive damage, and then to OHC loss. This may occur through inhibition of the glutamate-cystine antiporter that is responsible for exporting glutamate from cells and importing cystine, which is then converted to a key building block molecule needed for intracellular GSH synthesis. Loss of the GSH ultimately leads to the cell's demise because this protective molecule is key in the body's defense against oxidative stress. As discussed in the next section, other mechanisms may be responsible for noise-induced GSH depletion. These concepts of noise exposure–generated oxidative stress are summarized for the inner hair cells (IHCs) in **Fig. 32–4A** and OHCs in **Fig. 32–4B**.

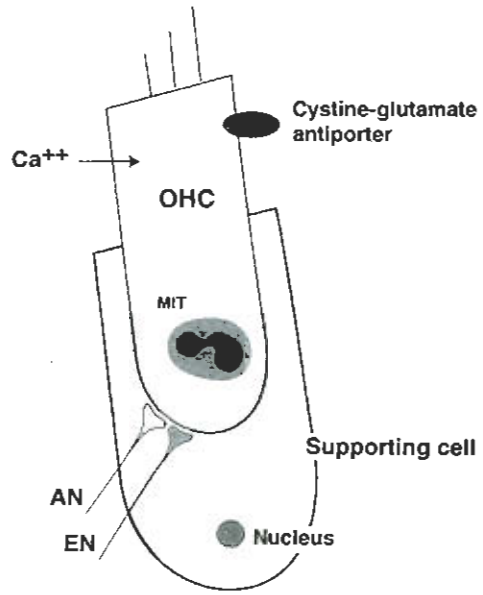
Glutathione Depletion

NIHL may, in part, be considered to result from the consequences of a temporary reduction in cochlear GSH in the face of oxidative stress. Because GSH is a key antioxidative stress metabolite, its depletion is a consequence of prolonged exposure to oxidative stress; however, its depletion also exacerbates the imbalance in the homeostasis of cochlear tissues caused by the ongoing oxidative injury. Thus GSH depletion is both a consequence and a cause of oxidative cell injury. GSH is one of the key cellular antioxidant molecules present within eukaryotic cells. It is a tripeptide composed of the amino acids glutamate, cysteine, and glycine. The rate-limiting enzyme for its synthesis is γ-glutamylcysteine synthetase, which is regulated by feedback inhibition by its end product, GSH. GSH acts as a free radical scavenger, as well as a detoxification molecule against hydrogen peroxides and other peroxides. GSH keeps vitamins C and E in their reduced active states, maintains the thiol moieties on proteins and peptides in a reduced state, and detoxifies xenobiotics enzymatically through the GSH-transferase family of enzymes or by a nonenzymatic action by forming conjugates. Oxidized (inactive) GSH (i.e., GSSG) is returned to the reduced (active) GSH state through GSSG reductase, and this reaction requires a molecule of the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH). Methods for increasing



1. Ischemia reperfusion
2. Excessive glutamate release
3. Increase in neuronal Ca^{++}
4. Enhanced nitric oxide synthase activity
5. Enhanced ROS generation (NO and related species)

A



1. Ischemia reperfusion
2. Excessive calcium influx
3. Hyperproduction of ROS by mitrochondria
4. GSH depletion (inhibition of cystine-glutamate antiporter)
5. Activation of phospholipase A_2
6. Activation of xanthine oxidase
7. Damage from NO or HNE

B

Figure 32–4 *Postulated generators of oxidative stress in the organ of Corti.* ***(A)*** *Inner hair cell (IHC): Excessive release of glutamate (Glu) from overstimulated inner hair cells activates (Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-or N-methyl-D-aspartate (NMDA)-s type afferent neuronal postsynaptic receptors. This leads to a massive influx of calcium and other ions into the dendrite, leading to the activation of calmodulin. Nitric oxide synthase (NOS) is then activated to produce nitric oxide (NO) from l-arginine (l-Arg) in conjunction with a variety of other reactive oxygen species. Ordinarily, after being transported into a supporting cell by the glutamate transporter (GLAST), Glu may be metabolized to glutamine (Gln) in a supporting cell by glutamine synthetase and recycled back into the inner hair cell to be resynthesized into Glu by phosphate-activated glutaminase (PAG) located in the mitochondria (MIT). However, the efflux of Glu may be in excess of what the supporting cell GLAST can metabolize. Oxygen deprivation secondary to ischemia reperfusion may play a role in several of the depicted changes. (Modified from Nordang L, Cestreicher E, Arnold W, et al. Glutamate is the afferent neuro-transmitter in the human cycle. et al. Acta Otolaryngol 2000;120:359–362.)* ***(B)*** *Outer hair cell (OHC): OHCs are separated from IHCs by the tunnel of Corti and have reduced numbers of glutaminergic synapses compared with those present at the bases of the IHCs. The cystine-glutamate antiporter is shown. Ischemia-reperfusion, increases in intracellular calcium, and hyperproduction of ROS by OHC mitochondria (MIT) may play a role in subjecting OHCs to oxidative stress. Inhibition of the cystine-glutamate antiporter may be a source of oxidative stress unique to the OHC. 4-hydroxy 2,3-nonenal (HNE) may act as a relatively stable toxic molecule generated by oxidative-stress and be able to diffuse between cells. AN, afferent neuron; EN, efferent neuron; NO, nitric oxide.*

cellular GSH include the administration of an esterified form of GSH, methionine; L-Nacetylcysteine (NAC); L-2-oxothiazolidine-4-carboxylate (OTC); or γ-glutamylcysteine.

Glutathionine is not cell permeable, and to have a protective effect against ROS formation and ROS damage, GSH must be inside the cell. The esterified forms of GSH (i.e., monoethyl ester and diethyl ester) are cell permeable and are highly effective as a treatment to prevent ROS damage within an oxidative stressed cell. The monoethyl ester is more stable than the diethyl ester of GSH and therefore a better protective molecule for long-term administration. Esters of GSH when placed on the round window membrane (RWM) can protect the cochlea from impulse and continuous noise damage. GSH precursor molecules, OTC, and NAC (a source of the GSH precursor molecule cysteine) have been shown to reduce noise-induced threshold shifts when administered systemically prior to either continuous exposure to noise or exposure to an impulse noise.

Increases in Intracellular Calcium

One theory of noise-induced cochlear injury holds that acoustic overexposure leads to an excessive influx of calcium ions into the hair cells, leading to injury and death of the affected cells. Ionic calcium plays an important, but as yet incompletely defined, role in cochlear hair cell physiology, including regulation of transduction, neurotransmitter release, OHC slow motility, and OHC adaptation. Calcium ions normally enter hair cells through L-type calcium channels and voltage-sensitive calcium channels.

During acoustic overexposure, harmful elevations in intracellular calcium may occur as a result of ROS-induced or micromechanically induced damage to hair cell plasma membranes and ROS-induced injury to calcium regulatory proteins, and organelles, such as mitochondria and mechanical stimulation. Increases in cellular calcium levels can then induce a cascade of events, including activation of phospholipases (damage cell membranes), protein kinase C (disrupt microtubules), proteases such as calpain (alter membrane permeability), and endonucleases (impair protein synthesis). Activation of these processes may lead to cell injury and/or death of the cell.

AMELIORATING NOISE-INDUCED HEARING LOSS THROUGH PHARMACOLOGICAL THERAPIES

Given the theories outlined in the preceding section, it follows that approaches to prevent or reverse acute NIHL should be designed based on known mechanisms of pathogenesis. Hence, approaches to mitigate mitochondrial injury, reduce glutamate excitotoxicity, replenish GSH, and reduce harmful intracellular calcium fluxes have all met with some measure of success (novel strategies are presented and discussed in the following section).

Protecting/Restorating Mitochondria

Seidman and Van De Water (2003) have shown that a mitochondrial metabolite known as acetyl-L-carnitine (ALCAR) was effective in reducing age-related hearing loss in a rat model of presbycusis. Age-related hearing loss is also thought to be related to the accumulation of cochlear damage from chronic oxidative stress. We have shown that ALCAR is also effective at reducing NIHL in a chinchilla model when given before and after intense, continuous noise exposure (**Fig. 32–5**). ALCAR supplies acetyl moieties and carnitine. These molecules enhance mitochondrial energy production and restore a key mitochondrial molecule known as cardiolipin, while restoring mitochondrial membrane integrity. This restores mitochondrial efficiency with a consequent reduction of mitochondrial free radical formation, preventing mitochondrial-induced apoptosis.

Prevention of the release of cytochrome c from damaged mitochondria is also an effective therapeutic approach to reduce NIHL that results from excessive noise exposure. Cytochrome c release from damaged mitochondria can be prevented by preventing pore formation (e.g., overexpression of Bcl-2 or Bcl_{xl} via a gene therapy vector) or interruption of the signaling cascade of JNK/c-Jun cell death signaling pathway (e.g., c-Jun antisense therapy) that damages the mitochondria of oxidatively stressed auditory sensory cells.

Reducing Glutamate Excitotoxicity

Several strategies are possible for reducing noise-induced glutamate toxicity, including countering the damaging ionic fluxes nonspecifically through magnesium supplementation, or more specifically, through the application of an antagonism of glutamate receptor–associated ionic channels. Specific glutamate receptor antagonism can be accomplished, and NOS can be inhibited. In addition, treatment with esterified GSH can ameliorate the toxicity associated with excessive glutamate release.

Carbamathione, a glutamate receptor antagonist, given systemically to chinchillas beginning prior to 6 hours of exposure to a 105 dB SPL 4 kHz octave band noise, reduced both hearing loss and hair cell loss (**Fig. 32–5**). The decrease in NMDA receptor binding by glutamate is thought to be due to a modification of the redox modulator site on the receptor. Many glutamate antagonists available to date are associated with undesirable side effects due to the excessive activity of some of these blocking agents. Carbamathione [*S*-(*N*,*N*-diethylcarbamoyl) glutathione, also known as DETC-GS], unlike classical glutamate antagonists that yield complete inhibition at the level of interaction with the receptor (e.g., CGS 19755) or directly at receptor-linked, calcium ion channels (e.g., phencyclidine or MK 801), is thought to impart its inhibitory effects via interaction with the redox modulatory site of the NMDA receptor. The latter type of interaction produces partial glutamate antagonism, is selective for the NMDA subtype of glutamate receptor, and should produce fewer unwanted side effects.

Reducing GSH Toxicity

Maintaining, enhancing, and restoring cochlear GSH levels have several potential advantages as a treatment strategy to reduce NIHL. First of all, GSH is a key intracellular antioxidant and inhibitor of stress-induced

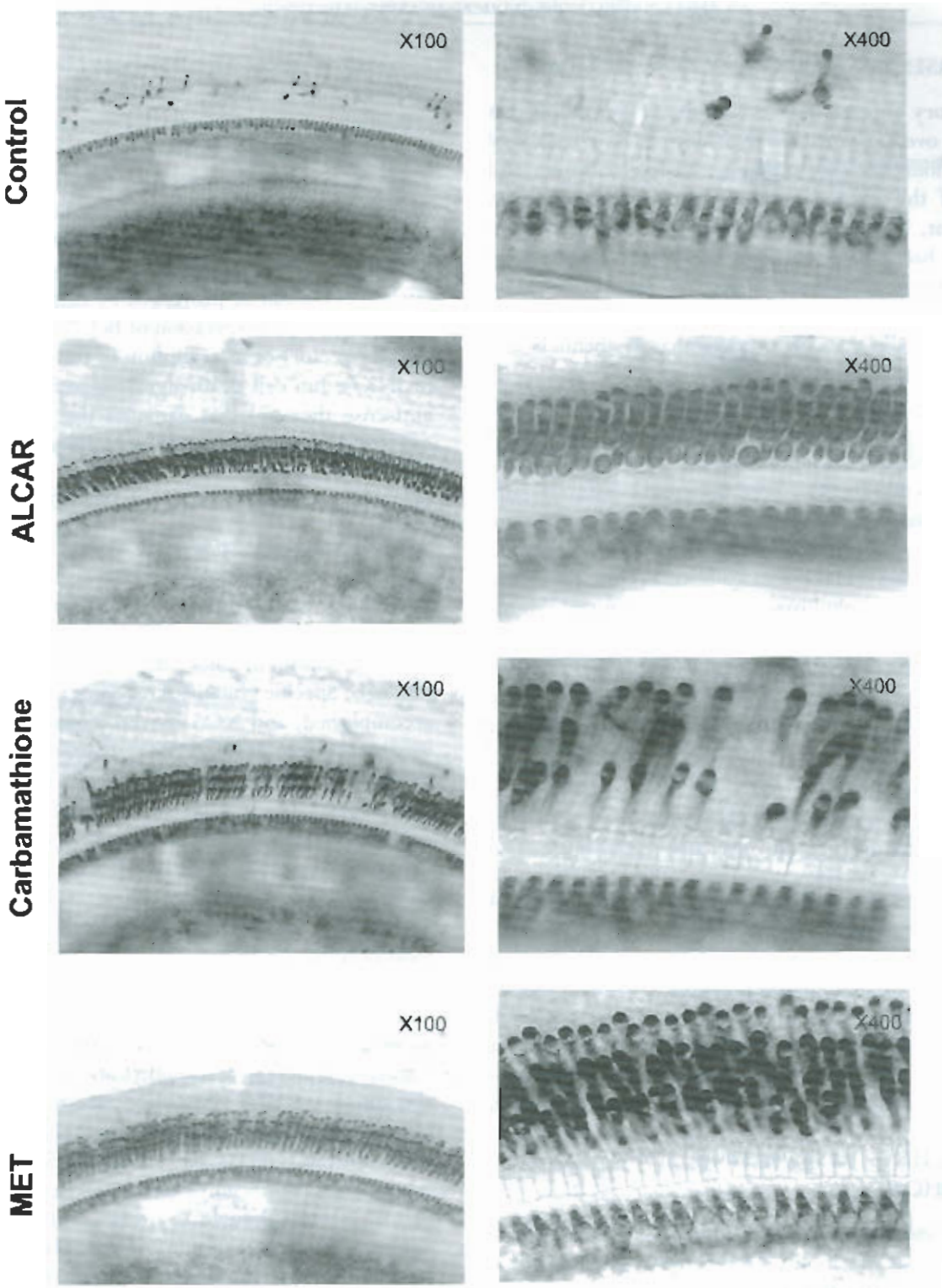


Figure 32–5 *Treatment of noise-exposed animals with either acetyl-l-carnitine (ALCAR) or methionine (MET) prevents loss of hearing and auditory hair cells. Photomicrographs are cochlear surface preparations, with the hair cells stained with sodium succinate histochemistry. Micrographs on the left are low-power (100×) and those on the right are high-power (400×) images of the 6 kHz regions of cochleas. All photomicrographs were taken from noise-exposed animals treated with either saline (control) or ALCAR, carbamathione, or MET, depicted in rows from top to bottom. The single row of inner hair cells (IHCs) is oriented toward the bottom, and the three rows of outer hair cells (OHCs) are toward the top of each micrograph. The control animal demonstrates an almost total loss of viable (stained) OHCs, with a scattered loss of IHCs in the sound trauma lesion site. In contrast, micrographs from MET- and ALCAR-treated noise-exposed animals demonstrate a single row of intact IHCs and three complete rows of viable OHCs, similar to the pattern of these sensory cells in non-noise-exposed cochleas (not shown). Carbamathione-treated animals demonstrated only partial protection of the cochleas from sound trauma with an intermediate level of OHC losses.*

apoptosis. GSH is an effective detoxifying molecule for lethal lipoperoxides, such as HNE, that may be produced as a consequence of excessive noise. Additionally, GSH can counter the harmful effects of glutamate excitotoxicity, mitochondrial injury, and excessive intracellular calcium fluxes. Thus GSH levels seem to be important in ameliorating the damage caused by all of the pathological mechanisms described for noise-induced, cochlear oxidative stress mentioned in this review. GSH is rapidly metabolized on its first pass through the liver and is poorly transported into most cells; it therefore has limited bioavailability.

GSH is not cell permeable and must be present within a cell to provide effective otoprotection; therefore, treatment with unmodified GSH is ineffective as a therapy. Fortunately, intracellular GSH levels may be enhanced through the use of a variety of GSH or cysteine precursors and cell-permeable forms (esters) of GSH. These agents, such as NAC, methionine, GSH esters (GSHe), and thiazolidine-related drugs, such as 2-oxothiazolidine-4-carboxylate, have in common that they are readily transported into cells and serve as intracellular sources of cysteine, which can be used by the cell to produce GSH, or in the case of GSH esters have a direct action. All have been shown to reduce

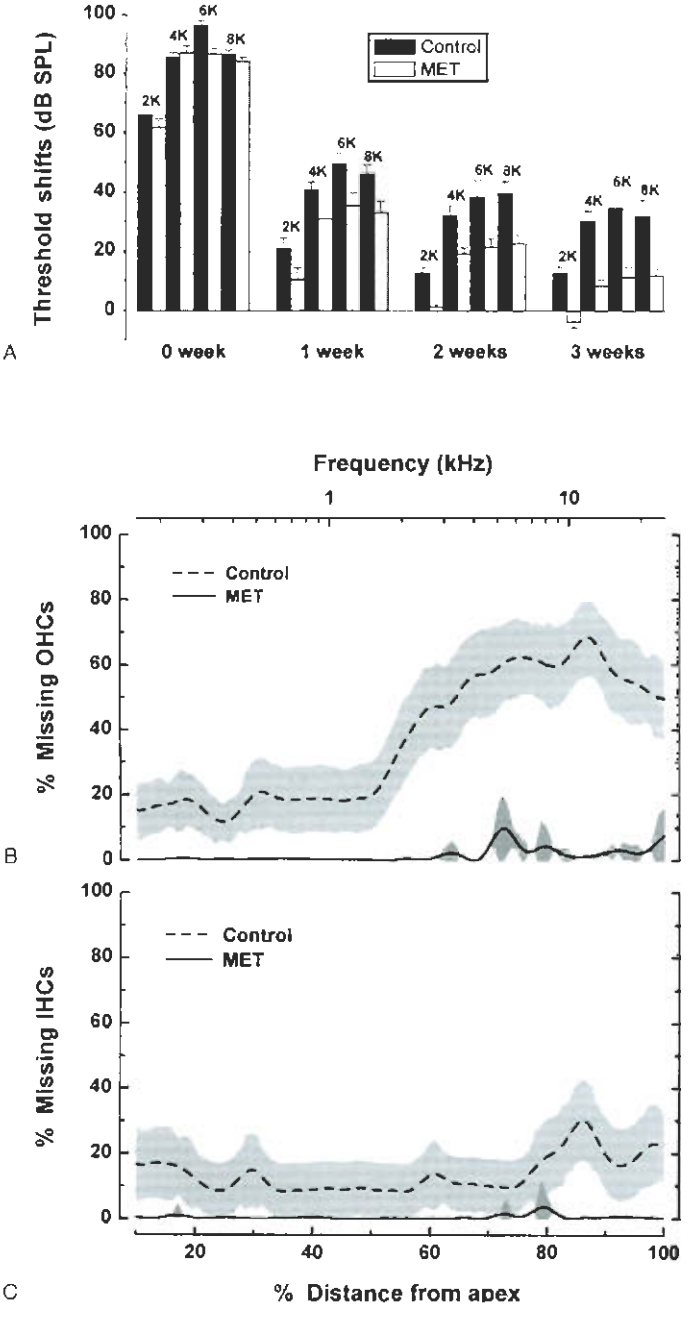


Figure 32–6 *Methionine (MET) treatment protects hearing from noise exposure–generated hearing loss and loss of auditory hair cells.* ***(A)*** *Auditory threshold shifts for saline-treated and MET-treated animals following exposure to a damaging level of noise. Means for auditory threshold shifts (dB SPL) plotted as a function of treatment group (saline control-noise and MET treatment-noise), time [0 (1 hour), or 1, 2, 3 weeks post-noise exposure], and by threshold test frequency for 2, 4, 6, and 8 kHz. Initial threshold shifts (week 0) ranged from ~62 to 87 dB for the MET-noise group, which were statistically similar to the saline-treated noise-exposed group ($p > .05$), except for 6 kHz, where the mean threshold was significantly less than that of controls ($p < .05$). An overall treatment effect for the MET-treated group was protective when compared with the saline-noise group ($p < .001$) for all test frequencies beginning at week 1. Error bars are ± standard error of means (SEM) Sample (n) size is 12 for all groups (12 ears, six animals).* ***(B)*** *Outer hair cell (OHC) cytocochleogram results. Depicted are mean values (continuous line) and standard error of the mean (shaded area) cytocochleograms for OHCs MET-pretreated noise-exposed cochleas (solid line) and saline-treated noise-exposed cochleas (dotted line), respectively. The y-axis depicts mean percent missing OHCs. The lower x-axis represents percent distance from the cochlear apex, and the upper x-axis depicts the associated frequency range of the cochleas in kHz. A very small loss of OHCs occurred in the low-dose MET-protected cochleas (<10%), whereas substantial OHC losses occurred in the saline-control noise-exposed group (average of ~60% for the 4–10 kHz region). These differences were significant ($p < .001$). Error bars are ± standard error of means SEM. Sample (n) size is 12 for all groups (12 ears, six animals).* ***(C)*** *Inner hair cell (IHC) cytocochleogram results. Illustrated are the mean values of IHC cytocochleograms with missing IHC percentages on the y-axis as a function of the measured percent distance from the cochlear apex. The associated frequency region of the cochlea in kHz is also plotted on the upper x-axis, and the percent distance from the cochlear apex is depicted on the lower x-axis. MET treatment (solid line) afforded significant protection of IHCs, as seen by a reduction to 5% or less of IHC loss, with MET treatment versus over 20% in the saline-treated animals ($p < .05$). Error bars are ± standard error of means SEM. Sample (n) size was 12 for all groups (12 ears, six animals).*

NIHL in laboratory studies. Results for protection against NIHL for methionine treatment are shown in **Figs. 32–6A, B,** and **C**.

Ameliorating the Effects of Increases in Intracellular Calcium

Approaches to reduce NIHL through the use of calcium channel blockers have been published in both the basic science and the clinical literature. The calcium channel blocker diltiazem was utilized pre- and postoperatively in a prospective, randomized, double-blind study (Heinrich et al., 1999; Maurer et al., 1995) as an approach to reduce noise trauma associated with otologic surgery. There was a tendency toward reducing noise-induced permanent threshold shifts in those patients given diltiazem, but this effect on NIHL did not reach statistical significance. Additional work in an experimental model has shown that diltiazem may modulate precipitable calcium in the hair cells in guinea pigs after noise exposure, as well as reduce damage to the organ of Corti caused by a sudden impulse noise. However, other studies in mice and gerbils found no protective effect from noise with either diltiazem or another calcium channel blocker, nimodipine. With regard to diltiazem, it may not achieve an adequate drug level in the inner ear due to issues related to its ability to cross the blood-cochlear barrier. At present, calcium channel blockers have not been found to be consistently effective in reducing NIHL in experimental models of sound trauma.

A variety of approaches can be imagined to reduce NIHL pharmacologically (see summary in **Fig. 32–7A,B**). Several GSH-restoring compounds are in clinical use at this time and are orally well tolerated with few side effects. These agents can be given in tablet form prior to loud noise exposure in the workplace or in a recreational setting. Recent evidence shows that NAC and MET, two molecules used for intracellular GSH synthesis, can reduce NIHL substantially when given as a treatment agent shortly after noise exposure. As previously mentioned, GSH has multiple positive effects, including protecting and restoring mitochondrial function, reducing the effects of glutamate excitotoxicity, reducing the injury associated with excessive intracellular calcium levels, and preventing stress-induced apoptosis. Because of these many positive effects, the strategy to reduce acute NIHL by augmenting inner ear GSH levels or direct treatment with an esterified form of GSH is quite appealing. An additional strategy of clinical utility may be to treat with the mitochondrial metabolite ALCAR. ALCAR has been shown to be effective in reducing NIHL when given around the time of the acute noise

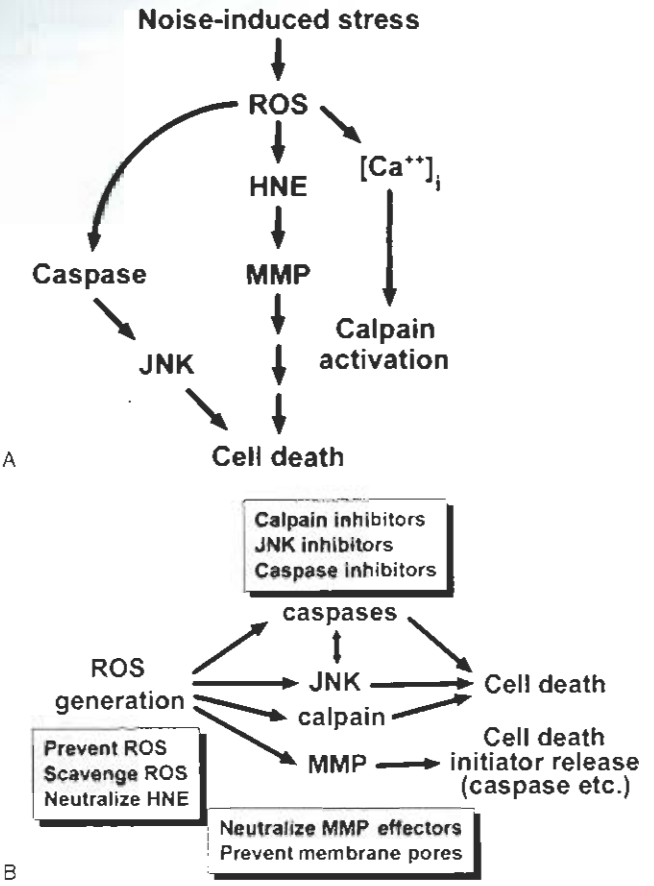


Figure 32–7 Noise-damage activation of cell death (apoptosis) within the cochlea. ***(A)*** *Cell death pathways. An outline of how noise-induced stress can lead to cell death in the cochlea. Multiple pathways are likely to be involved. Upstream events include the generation of reactive oxygen species (ROS) and perhaps a pathological increase in intracellular calcium. 4-hydroxy 2,3-nonenal (HNE) is likely formed downstream of the noise-damage-induced burst of ROS production. Increases in intracellular calcium also may occur at several points due to ROS-induced cell injury, including mitochondrial injury. Mitochondrial membrane permeabilization (MMP) is a central event in the cell death process. Caspases may be activated either following MMP or independent of MMP. Caspases may activate the c-Jun-N-terminal kinase and c-Jun (JNK/c-Jun) cell death pathway downstream of MMP or be activated independently and upstream of MMP. Caspase activation also can occur downstream of (JNK/c-Jun) activation (see* ***Fig. 32–3****). Upstream activation is depicted in this figure. Calpain activation, caspase activation, JNK activation, and MMP can all lead to cell death by interrelated and also by independent pathways.* ***(B)*** *Potential points of intervention for the treatment of NIHL and apoptosis of auditory sensory cells. Upstream points of intervention include the use of antioxidant and free radical scavengers to prevent the formation of ROS, to scavenge ROS, and to neutralize HNE. More downstream interventions include the use of inhibitors of calpain, JNK, and caspases. Another downstream strategy would be to neutralize MMP effectors and/or to prevent mitochondrial membrane pore formation.*

exposure. High dosage levels of ALCAR are approved for clinical application and have been used for a year or longer to treat chronic neurodegenerative diseases and diabetes. The use of glutamate antagonists shows promise; however, clinical side effects with these agents may limit their usefulness. Experimental evidence to date would suggest that calcium channel blockers are less likely to be effective clinically than the other agents already listed and discussed in this chapter.

Another set of interesting treatment strategies might be to treat the noise-injured cochlea with drugs that block programmed cell death pathways. While GSHe, MET, and perhaps ALCAR can all fulfill this role, other more specific apoptotic pathway inhibitors might be employed. For example, leupeptin, a calpain inhibitor, has been found to be somewhat effective in reducing noise injury to the cochlea; there are both pancaspase inhibitors and specific inhibitors of caspases that have yet to be tested as a protective therapy against NIHL. Investigators have published success in reducing NIHL in animal models by inhibiting steps in the mitogen activated protein kinase (MAPK) signaling pathway of apoptosis and more specifically, mixed lineage kinases (MLKs) that lead to the activation of the JNK/c-Jun segment of this pathway and also a peptide inhibitor that directly blocks the activation of the JNK molecule.

Finally, a future strategy for revising long-standing NIHL might involve regeneration of cochlear sensory hair cells coupled with the reestablishment of functional synapses. Birds have been shown to form new auditory hair cells through cell division of support cells followed by differentiation of new hair cells and support cells and/or transdifferentiation of existing support cells as sources of replacement hair cells. The newly formed replacement hair cells acquire new synaptic connections and restore auditory function. Mammals have not been shown to form new hair cells after injury in mature animals. This may be explained in part by the presence of cell proliferation inhibitors found to be active in the cells of the mature mammalian cochlea. Nevertheless, in the future it may be possible to override this block on proliferative regeneration through the use of trophic factors alone or in combination with inhibitors of cell proliferation inhibitors. Genes important for the genesis of hair cells in mammals, including *Notch*, *Math 1*, *Brn3.1* and many others, are just being discovered. Therefore, it may become possible to manipulate the expression of these genes or to deliver these genes to cochlear epithelial tissues to effect a hair cell regeneration response with return of function in humans. An initial study of *Math 1* gene therapy in a mammal suggests that this may be possible. This approach will require much additional research, and the possibility of the renewal of cochlear hair cells in arrears of damage and hair cell loss, although distant, seems real.

CONCLUSION

NIHL remains an important 21st century otolaryngology health problem responsible for permanent hearing loss in some 10 million individuals in the United States alone.

Current hearing conservation measures involving engineering, avoidance, and the use of HPDs remain important but have their limitations. A future comprehensive hearing conservation program may include pharmacological treatment to prevent and/or reverse acute NIHL. The "oxidative stress hypothesis" of noise-induced cochlear injury and loss of auditory sensory cells suggests a variety of pharmacological treatment strategies to ameliorate NIHL. These strategies include the oral administration of several different compounds before or after noise exposure. These compounds include agents to replenish cochlear GSH (e.g., NAC), esterified GSHe, mitochondrial protectants (e.g., ALCAR), glutamate toxicity antagonists, or calcium channel blockers, or agents to prevent noise-induced cochlear vasoconstriction. Additionally, specific programmed cell death pathway inhibitors (e.g., round window membrane application of c-Jun antisense oligonucleotide therapy or D-JNKI-1 treatment) may prove to be useful clinically. Finally, the ability to initiate auditory sensory cell regeneration through gene therapy looms in the future as a challenge, an exciting possibility, and a worthwhile goal to restore not only NIHL but also sensorineural hearing loss from other causes.

NOTE

The views expressed herein are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the United States government.

Suggested Readings

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Self-Test Questions

For each question select the correct answer from the lettered alternatives that follow. To check your answers, see Answers to Self-Tests on page 716

1. Limitations of mechanical personal hearing protection devices include
   - A. Noise energy exceeds protection afforded by the device.
   - B. Transmission of damaging acoustic energy through the skull
   - C. Improper fit of the device
   - D. Limitations in protection from different frequencies of noise
   - E. All of the above
   - F. A and C only
2. The oxidative stress hypothesis of noise-induced hearing loss
   - A. Is supported by the finding of noise-induced changes in cochlear glutathione (GSH), lipid peroxidation products, antioxidant enzyme activity, and free radical concentrations
   - B. Predicts that oxidative injury is the major cause of cochlear damage regardless of the degree of sound pressure level exposure
   - C. Is consistent with the finding that agents that increase cellular GSH decrease noise-induced cochlear damage
   - D. Is contradicted by the finding of the activation of programmed cell death pathways in the cochlea after intense noise exposure
   - E. A and C only
   - F. B and D only
   - G. A through D
3. Involvement of mitochondria in noise-induced cochlear injury can be described by which of the following?
   - A. Mitochondria are a major source of oxidative stress.
   - B. Mitochondria play an important role in the initiation of cell death processes after acoustic exposure.
   - C. Mitochondria are insensitive to glutamate levels induced by noise.
   - D. Mitochondria furnish their own GSH and are resistant to GSH depletion.
   - E. All of the above
   - F. A and B only
4. The time from acute narrow-band noise exposure to the loss of the majority of outer hair cells and establishment of a permanent hearing loss is best measured in
   - A. Minutes
   - B. Hours
   - C. Days
   - D. Weeks
   - E. Months